well as determining how their interaction regulates differentiation. Multiparameter fluorescence detection nowadays allows direct observation of molecular processes at the single molecule level.
The obtained evolutionary conserved multi-protein complex is located in the subapical region (SAR) of embryonic epithelia and plays a central role in the maintenance of epithelial cell polarity, morphogenesis and survival of photoreceptor cells in Drosophila melanogaster. The complex is composed of the four proteins DPATJ and DLin-7, Stardust (Sdt), and Crumbs (Crb). The scaffold protein Sdt contains two subsequent L27 modules, which mediate the interaction with DPATJ and DLin-7 through their L27 domains. The trans-membrane protein Crb binds to the PDZ domain of Sdt with its cytoplasmic tail.
Here we use single-molecule fluorescence resonance energy transfer (FRET) in order to understand better quantitative parameters and spatial dynamics of the complex. We show that free DLin-7 exists in two major conformations, a high-FRET, folded state, and a second low FRET, unstructured state, which allows us to assign the L27 domain in DLin-7 as an "extended" disordered region.
Upon formation of a complex with Stardust, the proportion of folded DLin-7 molecules increases. Depending on the Stardust/DLin-7 ratio different heteromers are formed. The presence of DPATJ further increases the fraction of DLin-7 bound to Stardust, indicating that the L27-domains of all three proteins contribute to a positive cooperativity. Thus, L27 domains are versatile modules ideally suited to provide flexibility of protein complexes.

368-Pos Board B247
Conformational Flexibility of the GM2 Activator Protein Loop Regions Investigated By Site Directed Spin Labeling EPR Spectroscopy
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The GM2 activator protein (GM2AP) is an essential component in the degradation pathway of neuronal gangliosides. GM2AP is a required accessory protein for the hydrolytic conversion of GM2 to GM3 by a water soluble hydrolase. The X-ray structure of GM2AP reveals a β-cup topology with multiple conformations of the protein within the unit cell. Because the crystal structures show different conformations of the putative membrane binding loops, we have utilized site-directed spin labeling to investigate conformational flexibility of these loops for protein in solution and bound with GM2 ligand. As such, a series of single and double CYS mutants (still with original 8 CYS in 4 disulfide bridges) have been generated and spin labeled with MTSL. EPR spectra of spin labeled GM2AP were collected with and without GM2 ligand, and no significant changes in the EPR lineshape were seen. EPR spectra were simulated for spin labels located in the loop regions and reveal multiple component fits, while those in the backside of the β-cup beta strands have single component fits. For certain sites in the mobile loops, spectra were acquired as a function of temperature. From these lineshape simulations, the activation energy for the conformational change has been determined. The SDSL EPR results indicate that the multiple conformations observed in the crystallographic unit cell are populated in solution and represent conformational flexibility of the protein; which is not necessarily altered by binding to lipid ligands. Additionally, spin labeled protein was analyzed by mass spectrometry to confirm proper formation of the four disulfide linkages and addition of only one spin label at the mutant cysteine.

369-Pos Board B248
Single-Molecule Protein Conformational Dynamics and Molecular Interaction Dynamics under Enzymatic Reactions
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Enzymatic reactions are traditionally studied at the ensemble level, despite significant static and dynamic inhomogeneities. Subtle conformational changes play a crucial role in protein functions, and these protein conformations are highly dynamic rather than being static. Protein-molecular interactions define the enzymatic reaction potential surface, pathway, and dynamics. The single-molecule protein-protein interaction dynamics reveals the nature of the molecular complex formation and recognition that are critical for an enzymatic reaction to occur. We applied AFM-enhanced single-molecule spectroscopy to study the mechanisms and dynamics of enzymatic reactions involved with kinase and lysozyme proteins. Enzymatic reaction turnovers and the associated structure changes of individual protein molecules were observed simultaneously in real-time by single-molecule FRET detections. We obtained the rates for single-molecule conformational active-site open-close fluctuation and correlated enzymatic reactions. We have demonstrated a specific statistical analysis to reveal single-molecule FRET anti-correlated fluctuations from a high background of fluorescence correlated thermal fluctuations. Our new approach is applicable to a wide range of single-molecule FRET measurements for protein conformational changes under enzymatic reactions.

370-Pos Board B249
Slippage Between Noncovalently Bound Filaments Of Self-assembling Peptide
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Much attention has been given to the rupture of noncovalent chemical bonds in protein dynamics, and Bell's model has become widely used. In many cases, however, unbinding or unfolding requires that multiple energy barriers be overcome in parallel, in coordinated failure events. We examine one such system, slippage between ß-sheet filaments of the self-assembling peptide RAD16-II ([RARADADA]2). RAD16-II forms amphiphilic ß-sheet filaments, with alanine side chains forming the hydrophobic surface. In an aqueous environment, filaments are found in pairs, with their hydrophobic faces placed together. We examine slippage between two filaments using steered molecular dynamics simulations. We observe that alanine side chains on one ß-sheet filament form a rectangular array, and the alanine side chains of the opposing sheet occupy the interstices. For slippage to occur, these methyl groups must jump from one interstice to the next. Since the alanines in one ß-sheet are elastically linked, this failure occurs in a cooperative manner. Slippage of a single alanine side chain correlates with slippage of its immediate neighbors, and a dislocation propagates across the bound surface within a few picoseconds. We present a one-dimensional, coarse-grained model based on Langevin dynamics, that incorporates the basic elements of this system: multiple elastically linked particles each residing in an energy well and overcoming an energy barrier under applied force. The coarse-grained model shows good agreement with molecular dynamics results and provides a useful platform for studying coordinated failure events. [Supported by the NHLBI, EB003805.]

371-Pos Board B250
Multiple Channels of Structural Relaxations in Functional Proteins
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We elucidate the physics of the dynamical transition via 15 ns long molecular dynamics simulations at a series of temperatures (spanning 160 - 280 K) where the protein retains its native structure. By tracking the energy fluctuations, we show that the protein dynamical transition is marked by a cross-over from a piecewise stationary to stationary set of processes that underly the dynamics of protein motions in the water environment.
We find that a two-time-scale function captures the non-exponential character of backbone structural relaxations. One is attributed to the collective protein motions and the other to local relaxations. The former is well-defined by a single-exponential, nanosecond decay that is operative at all temperatures. The latter, on the other hand, is described by a large number of single-exponential motions that display a distribution of time-scales. Though their average remains on the order of 10 ps at all temperatures, the distribution markedly contracts with the onset of the dynamical transition. Interestingly, the collective motions are shown to impose bounds on the time-scales spanned by the local dynamical processes, although they are not directly involved in the transition.
The piecewise stationary character below the transition implicates the presence of a collection of sub-states whose inter-communication is restricted. The ineffectiveness of these sub-states to influence the overall relaxation time is shown to require a wide distribution of local motion time-scales, extending well beyond that of nanoseconds. At physiological temperatures, on the other hand, local motions are confined to time-scales faster than nanoseconds. This relatively narrow window makes possible the appearance of multiple channels for the backbone dynamics to operate, providing alternative routes for protein functionality.

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Solvent Bridging Determines The Molecular Architecture Of The Unfolding Transition State Of A Protein
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Protecting osmolytes are ubiquitous in nature, where they play a vital role stabilizing intracellular proteins against adverse environmental conditions. While the solution thermodynamics of protein/osmolyte mixtures has been well characterized, information is lacking on how osmolytes influence the transition state structure and dynamics of proteins. Here we demonstrate a combination of single molecule force-clamp spectroscopy and solvent substitution that directly identifies the role of protecting osmolytes in the unfolding transition state structure of a protein. We measure the effect of osmolyte substitution on the rate of forced unfolding the I27 titin module. From the force dependency of the unfolding rate for each osmolyte we determine ΔG_U, the height